

Serotonin signaling and neuronal excitability in the inflamed colon: too much of a good thing

Gary M. Mawe, PhD
The University of Vermont

Collaborators: Keith Sharkey, Peter Moses, Michael Gershon

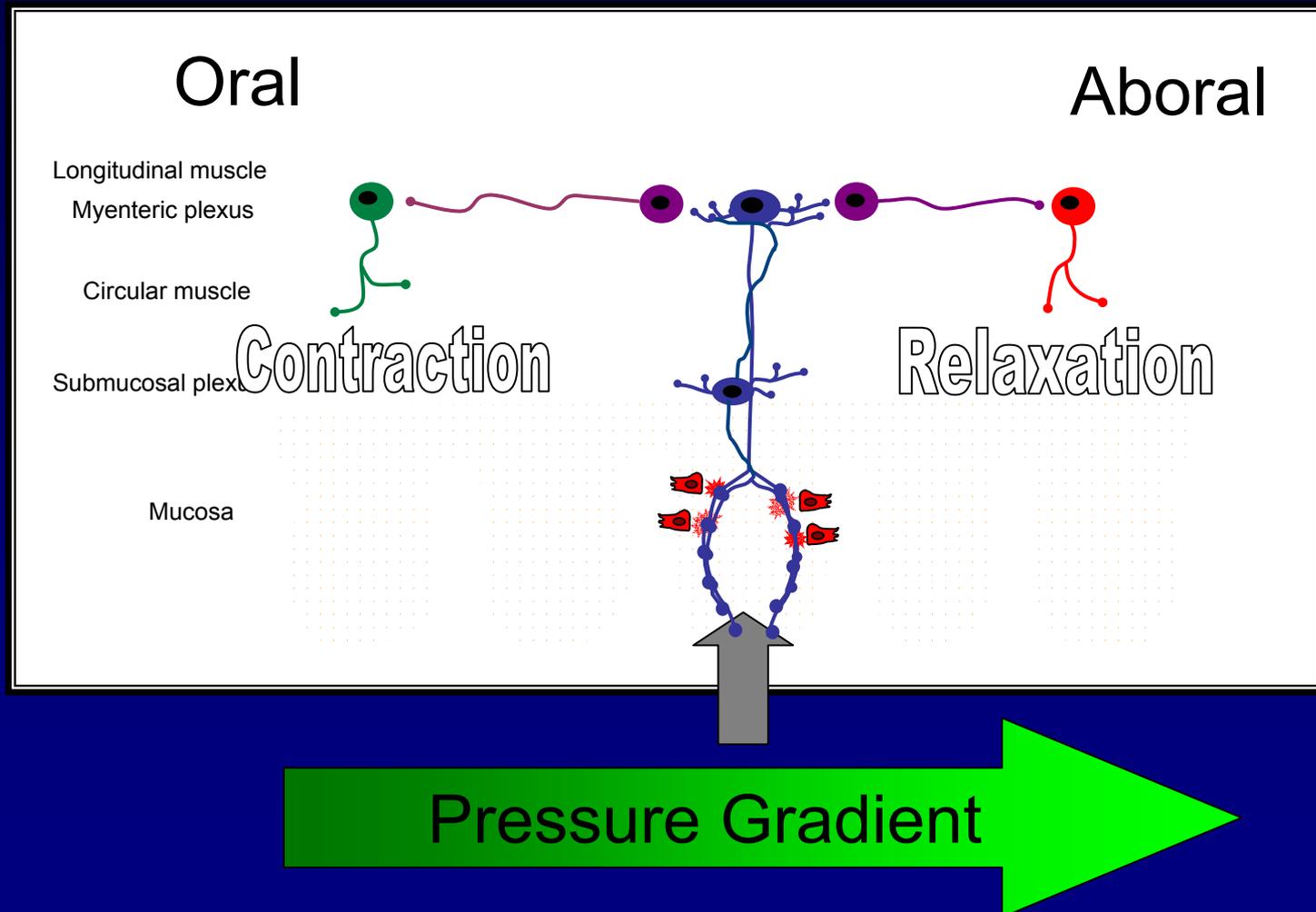
Postdoctoral & GI Fellows: David Linden, Jodie Sampson, &
Jason Chen

PhD and MD Students: Matt Coates, Jen O'Hara, Brian Manning
& Julie Simpson

Technicians: Christine Mahoney, Matt Salter

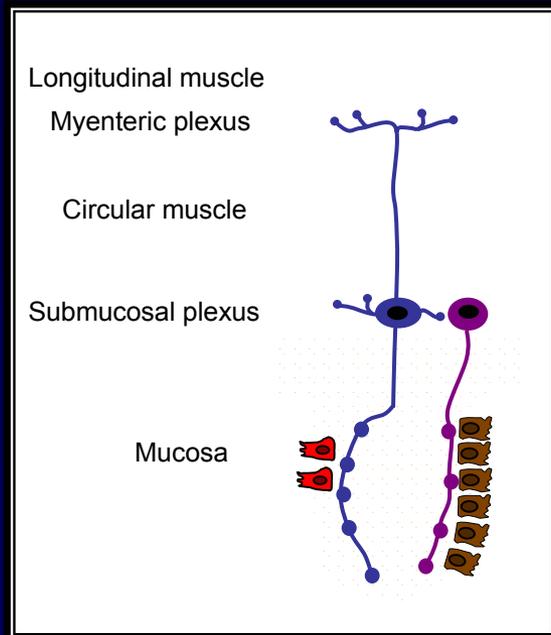
Student Researchers: Ciara Bannon

Intrinsic reflex circuits exist in the gut

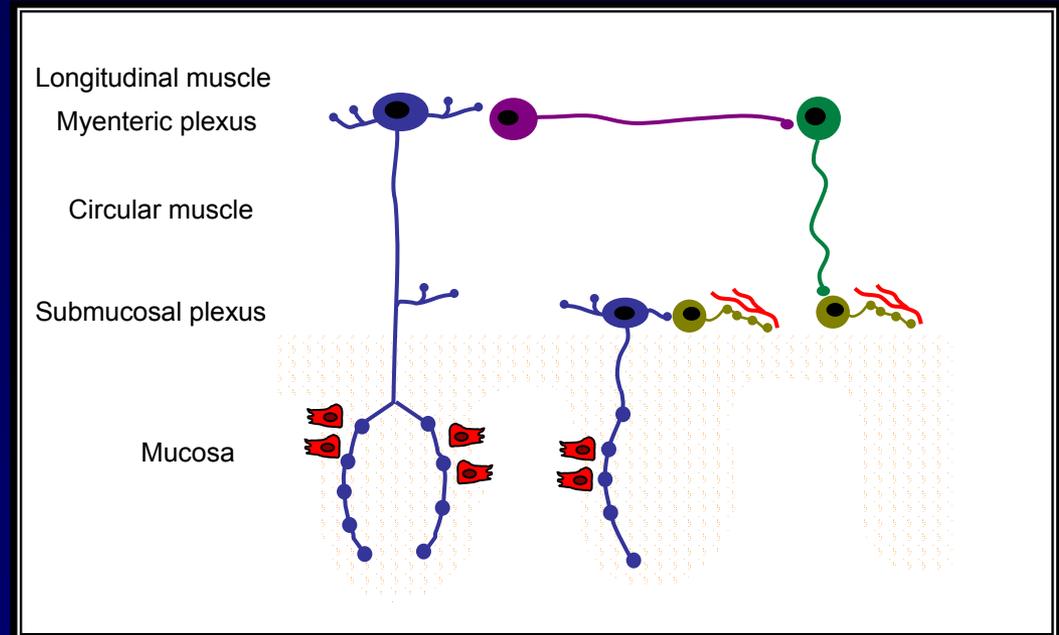


Other intrinsic reflexes in the gut

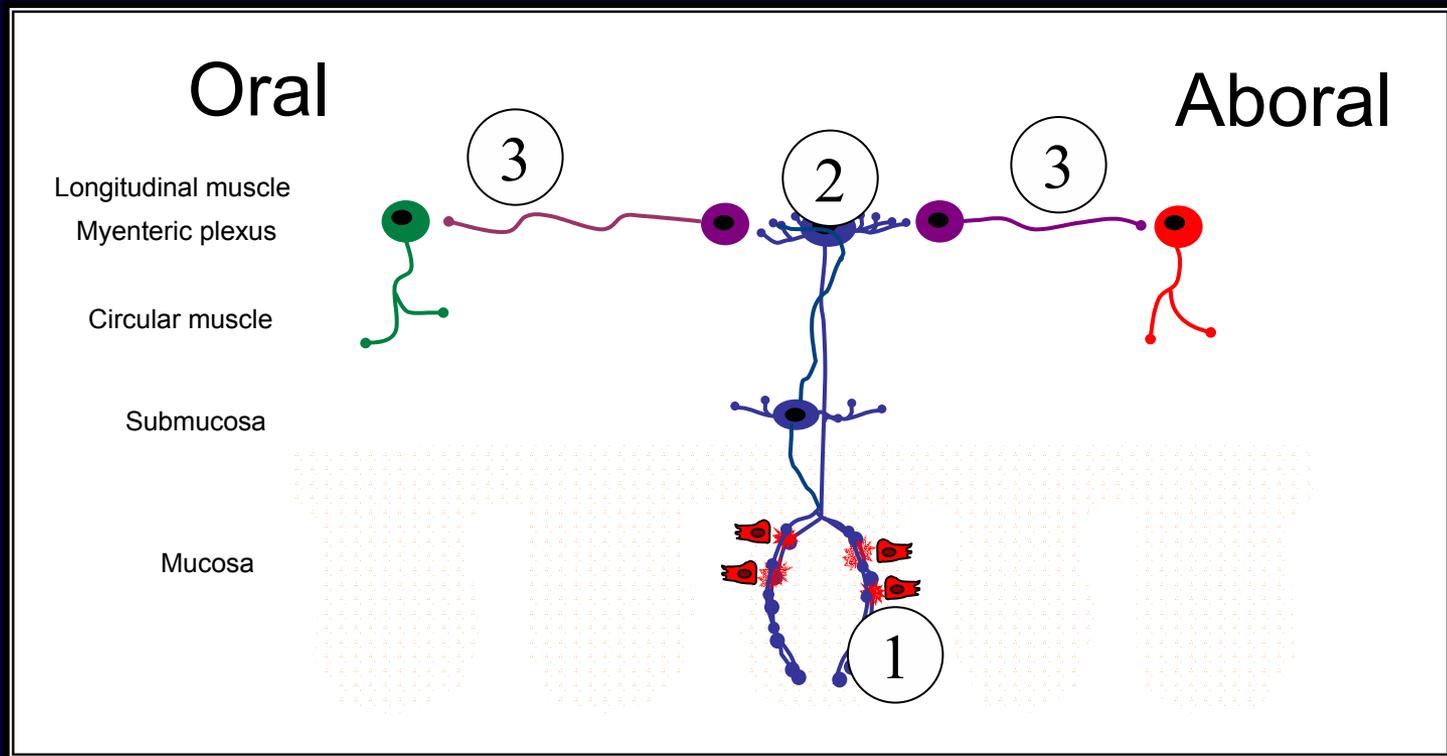
Mucosal secretion



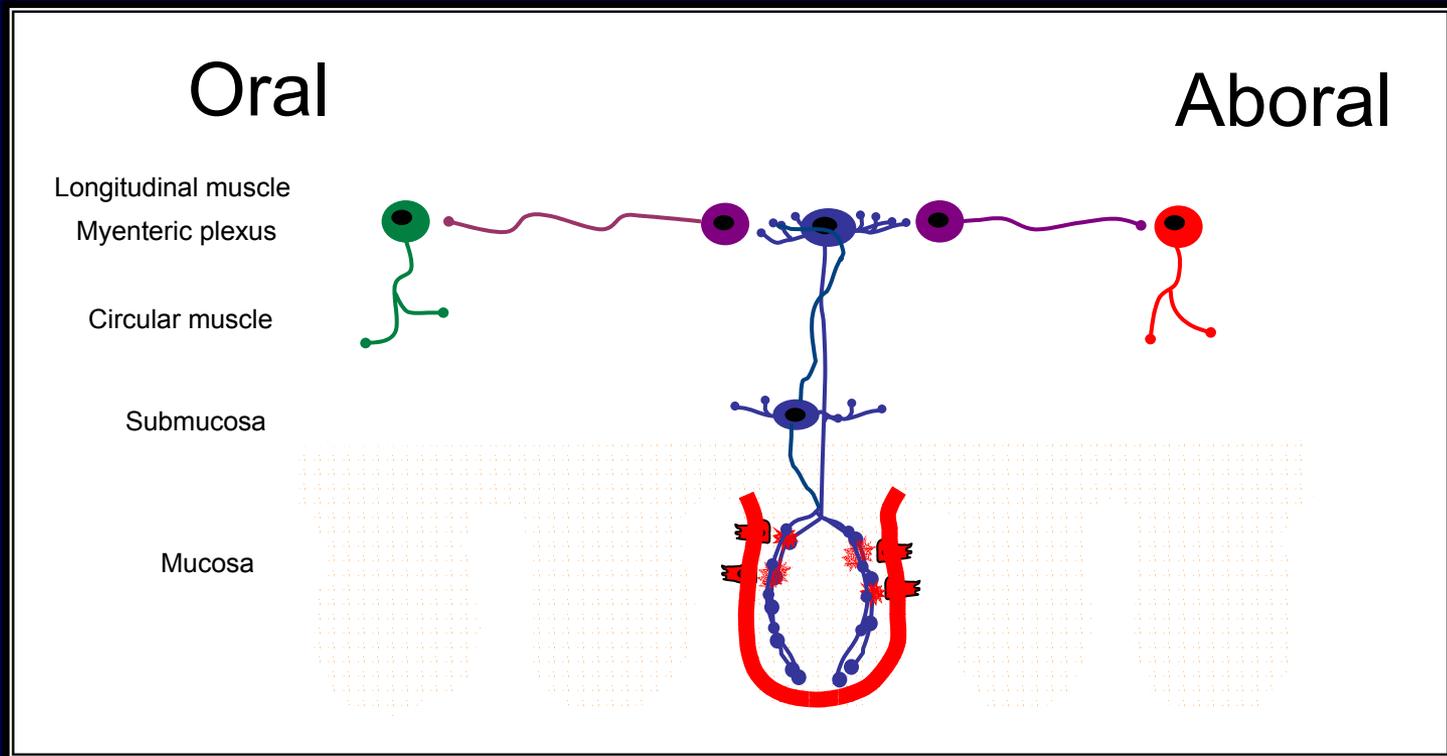
Vasodilation



Potential sites of changes in reflex circuitry



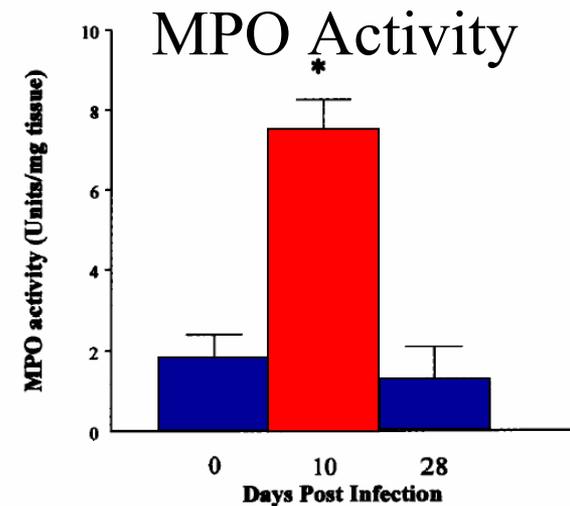
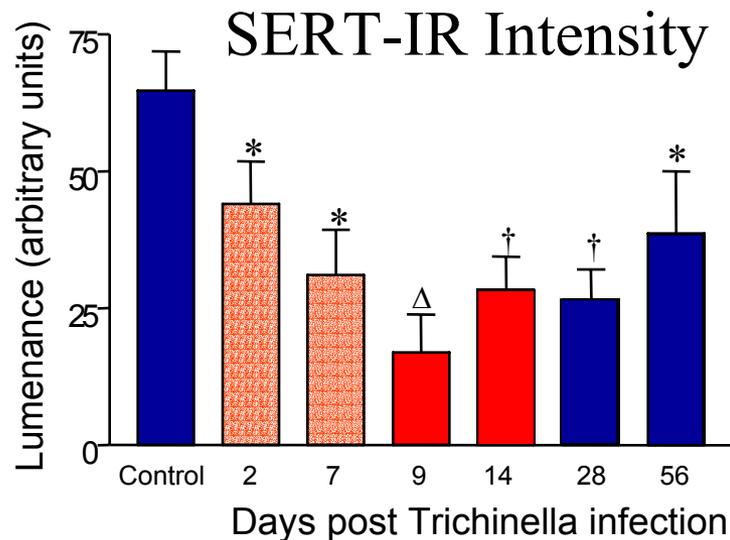
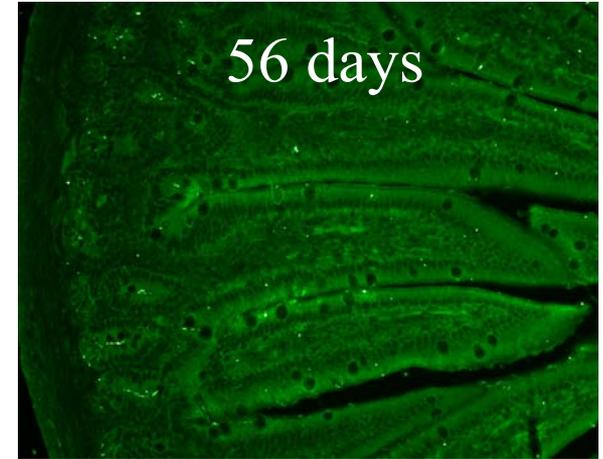
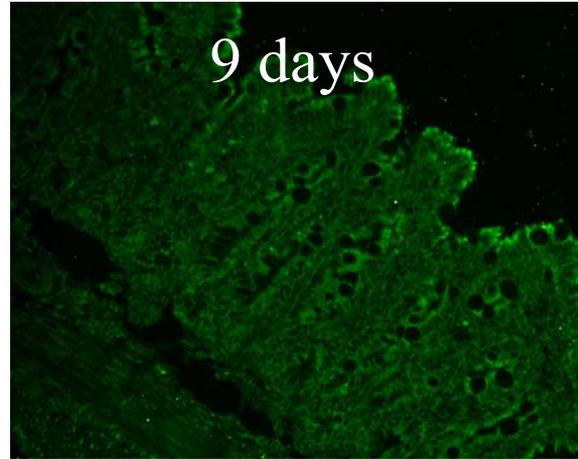
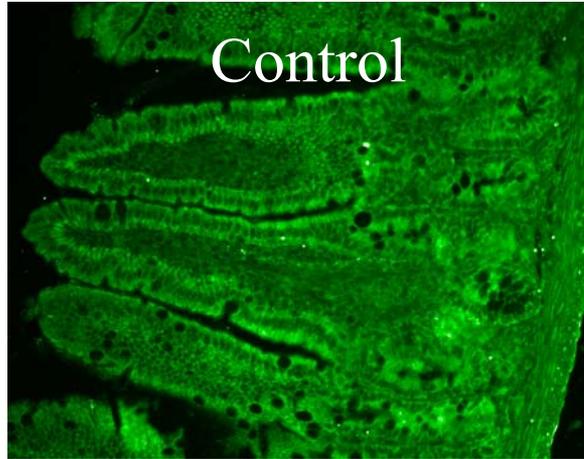
Potential sites of changes in reflex circuitry



Serotonin availability is increased in guinea pig and mouse TNBS-enteritis

	5-HT content	EC cell counts	5-HT release	5-HT transporter	References
Guinea pig TNBS-colitis	↑	↑	↑	↓	Linden et al. (2003) AJP-GI 285:G207-G216
Guinea pig TNBS-ileitis	↑	↑	↑	↓	O'Hara et al., in revision
Mouse TNBS-colitis	↑	?	?	↓	Simpson et al., unpublished
Mouse Trichinella-ileitis	?	↑	?	↓	Spiller et al., unpublished

SERT-IR is persistently decreased in mice following *Trichinella* infection



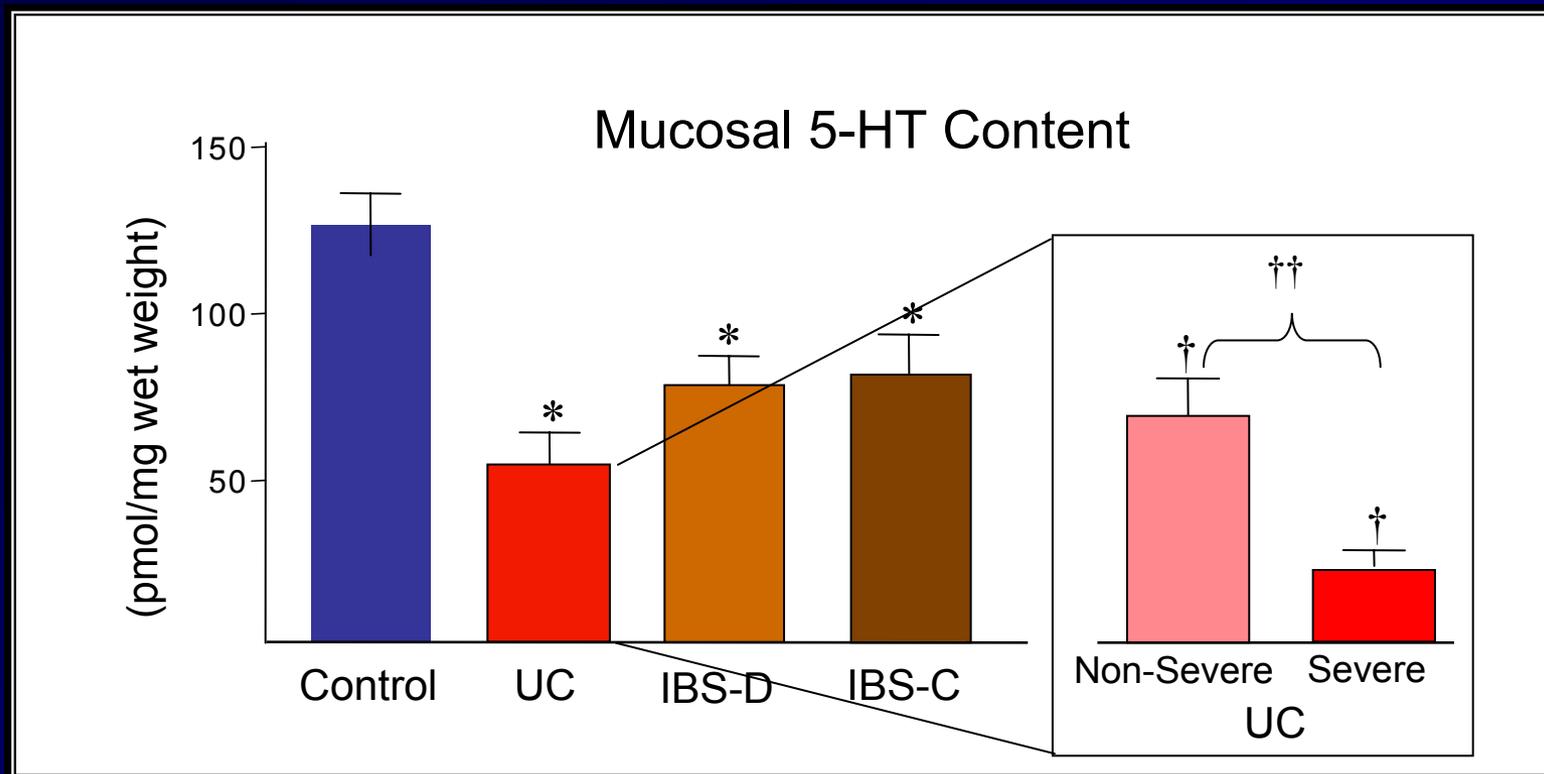
From Barbara et al., *Gastroenterology* 1997;113:1224-1232

Evaluation of the Key Elements of 5-HT Signaling in IBS and IBD

- Single center evaluation of normal controls (screening colonoscopy) and volunteers with IBS-C, IBS-D, UC. Screened by phone for medical history. Charts were reviewed as needed. Stringent diagnostic criteria were adhered to.
- Tissue obtained endoscopically using large capacity biopsy forceps.
- Measurement of 5-HT content and release, SERT immunoreactivity and mRNA, in the biopsy derived tissue.

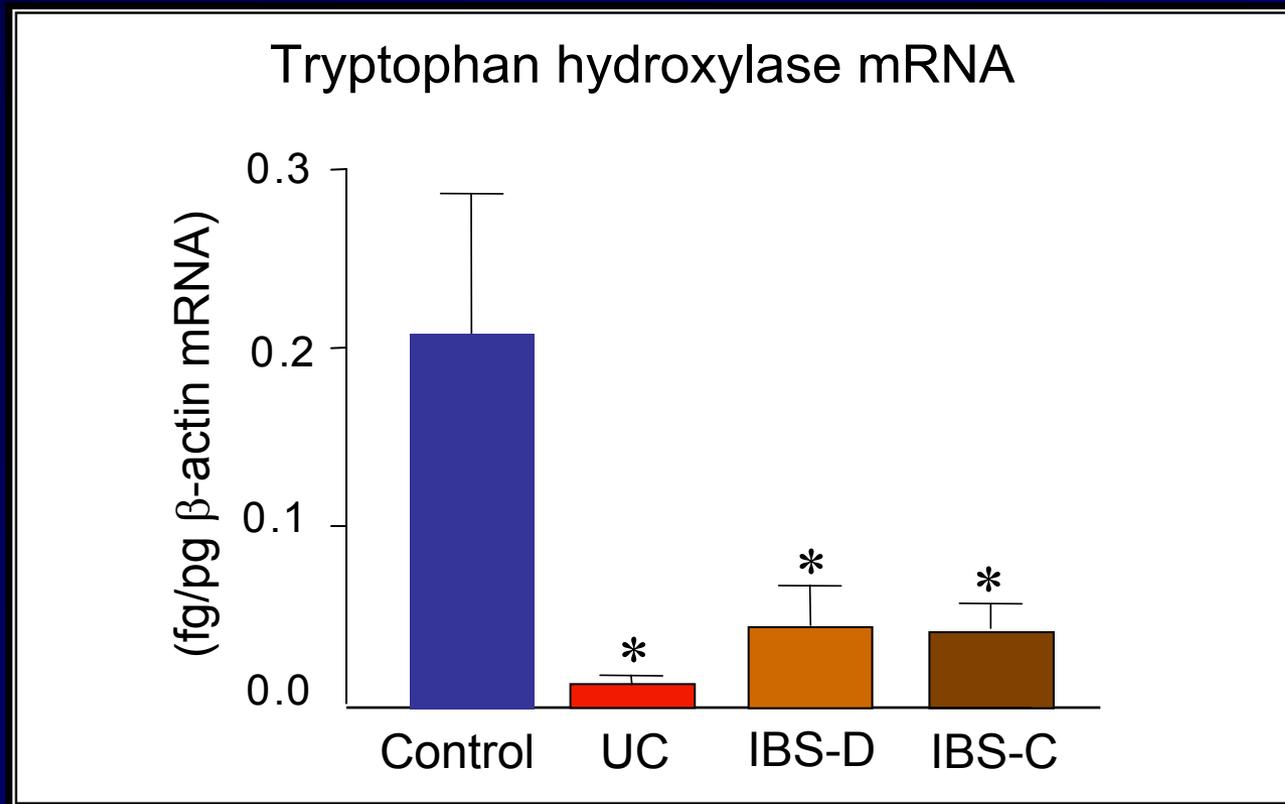


5-HT Content is Decreased in UC and IBS



*, different from control
†, different from control
†† different from one another

5-HT Synthesis is Decreased in UC and IBS



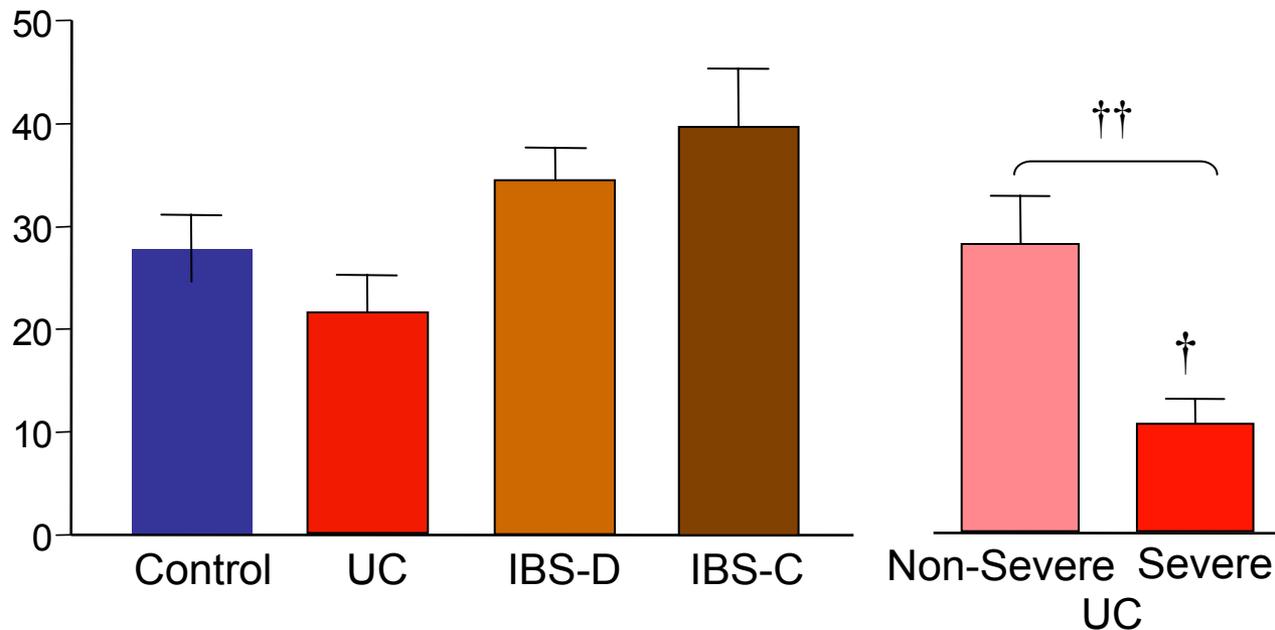
**, different from control*

no significant difference between severe and non-severe UC

Coates et al., In Press, Gastroenterology

EC cell numbers are decreased in UC

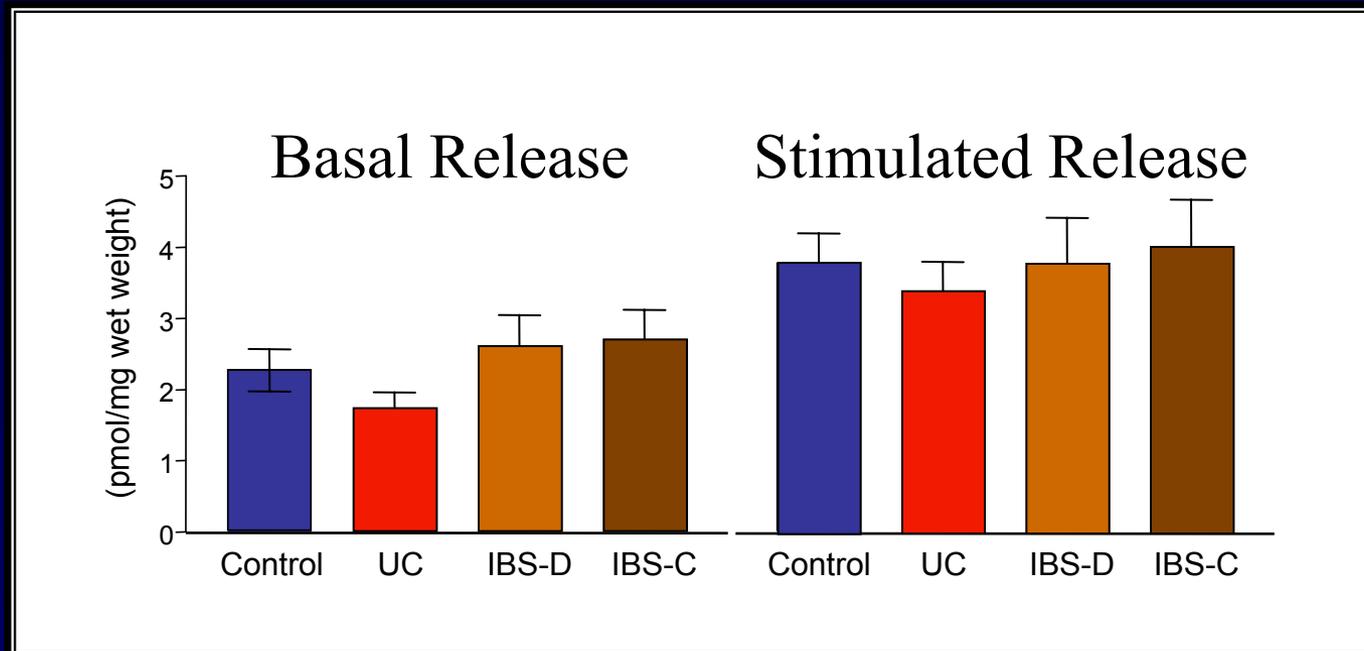
EC cells per mm muscularis mucosa



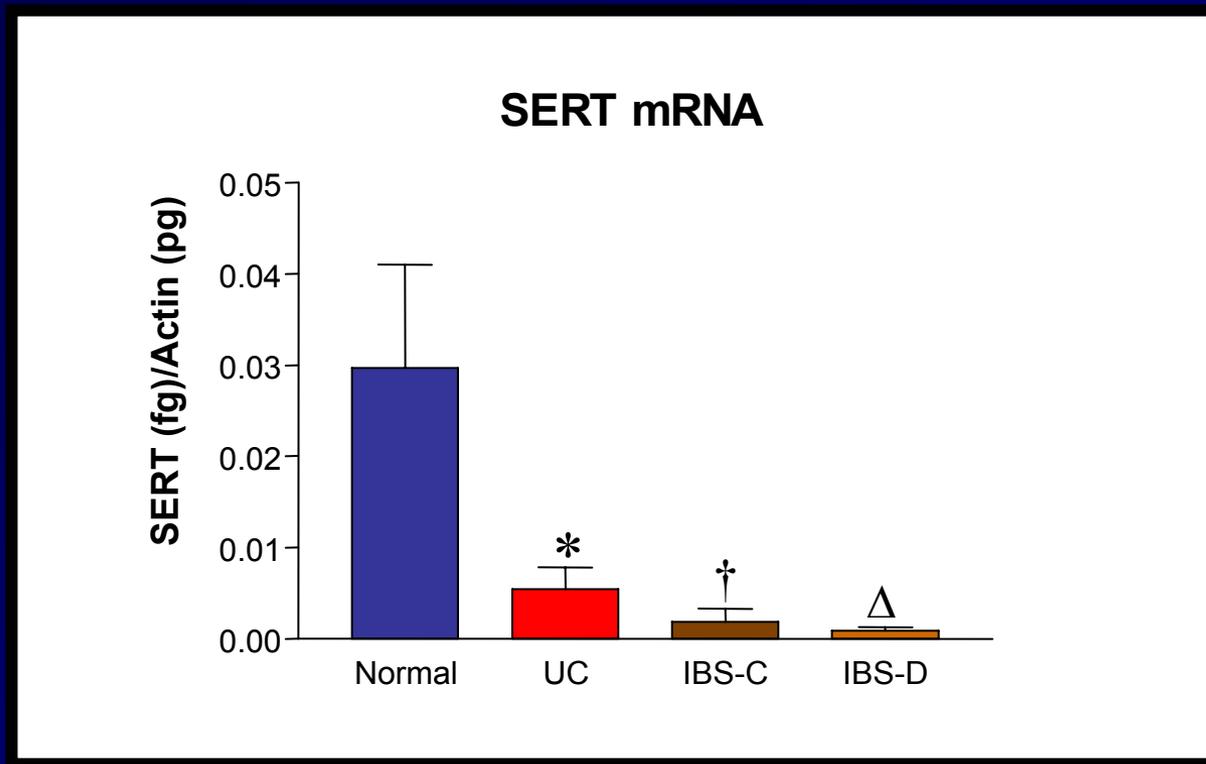
Human rectal biopsy
Chromogranin A
Serotonin + Chromogranin A
Yoyo, a nucleic acid stain

†, different from control; †† different from one another

5-HT release is not altered in IBD or IBS



SERT mRNA is decreased in IBD and IBS



n=18-26 per group

** $p < 0.05$, † $p < 0.01$, Δ $p < 0.001$ relative to control*

Coates et al., In Press, Gastroenterology

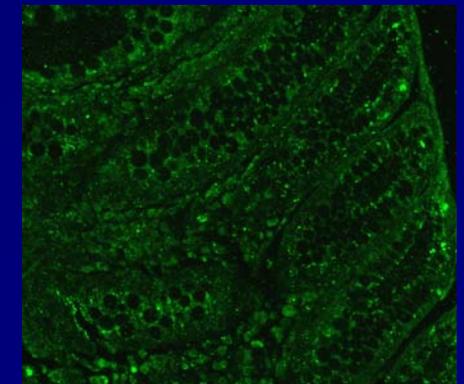
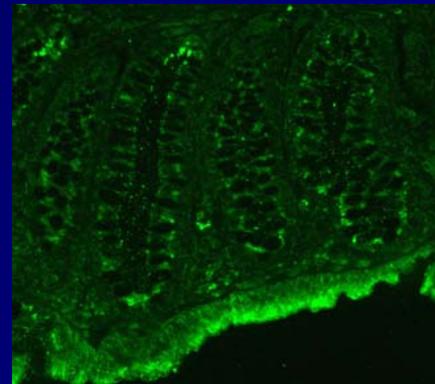
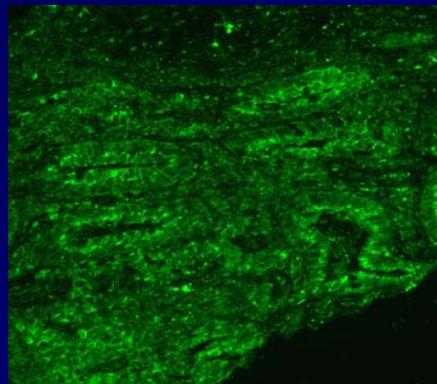
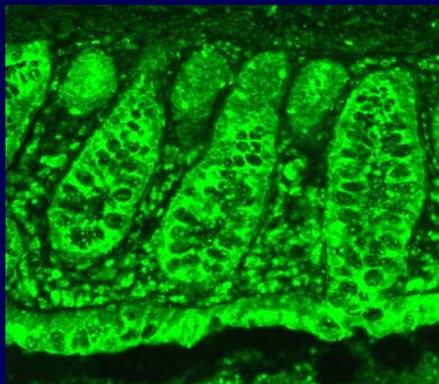
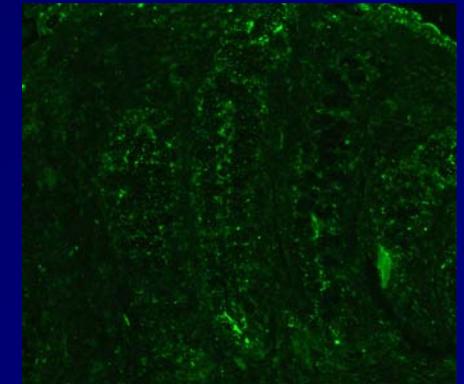
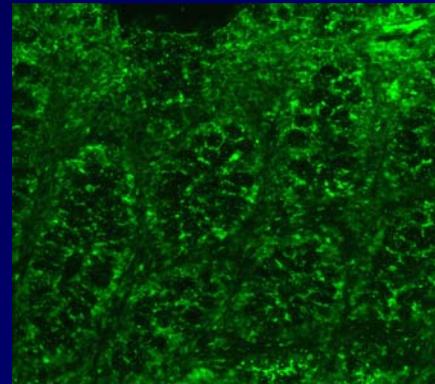
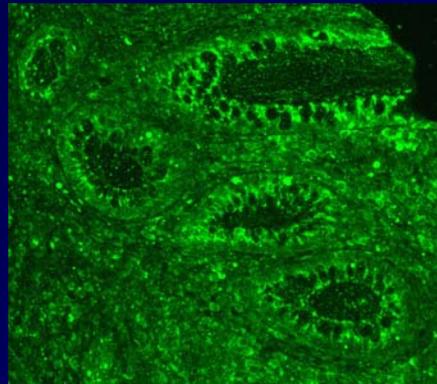
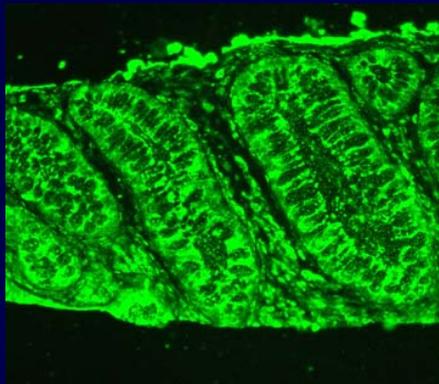
SERT-immunoreactivity in Human Rectal Biopsy Samples

Control

UC

IBS-D

IBS-C



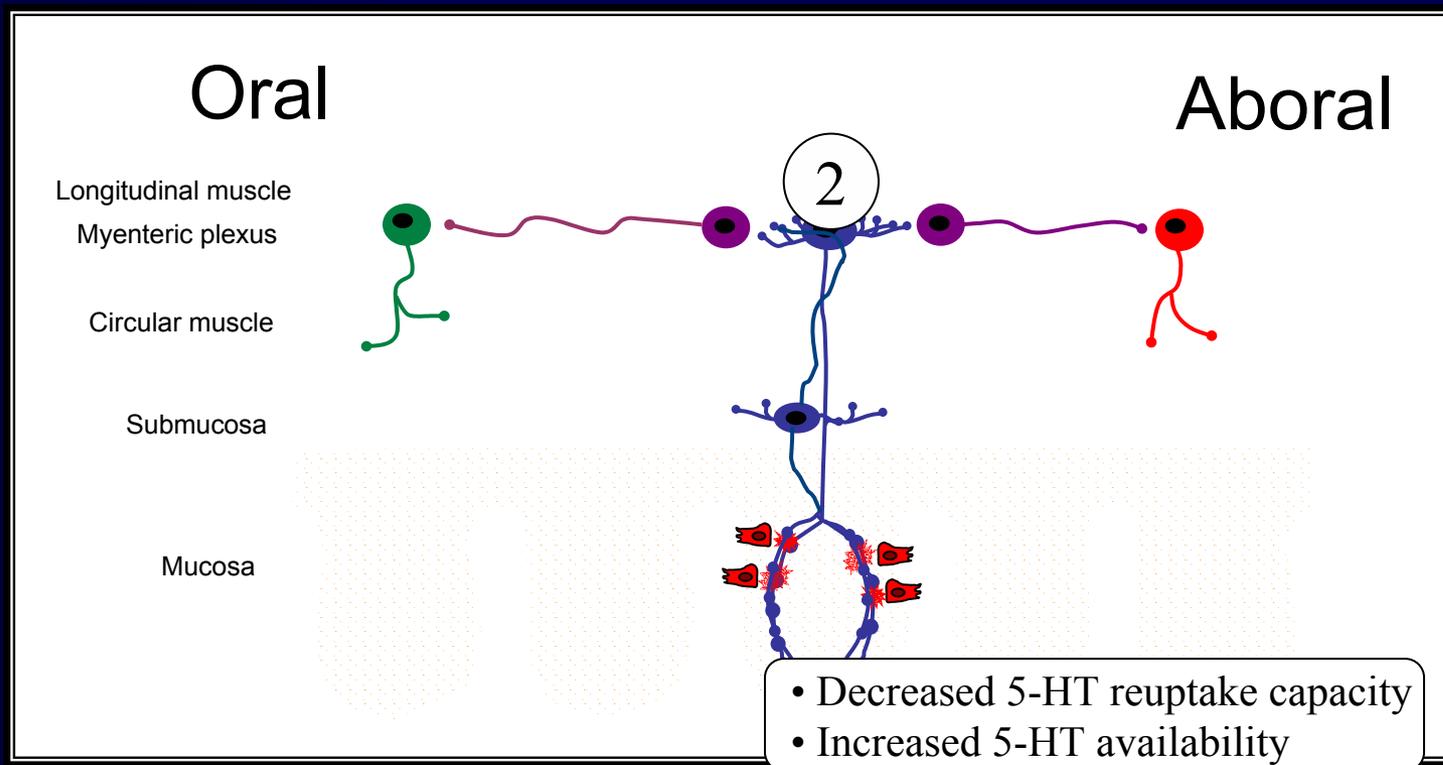
Summary of 5-HT properties in inflamed intestines

Animal

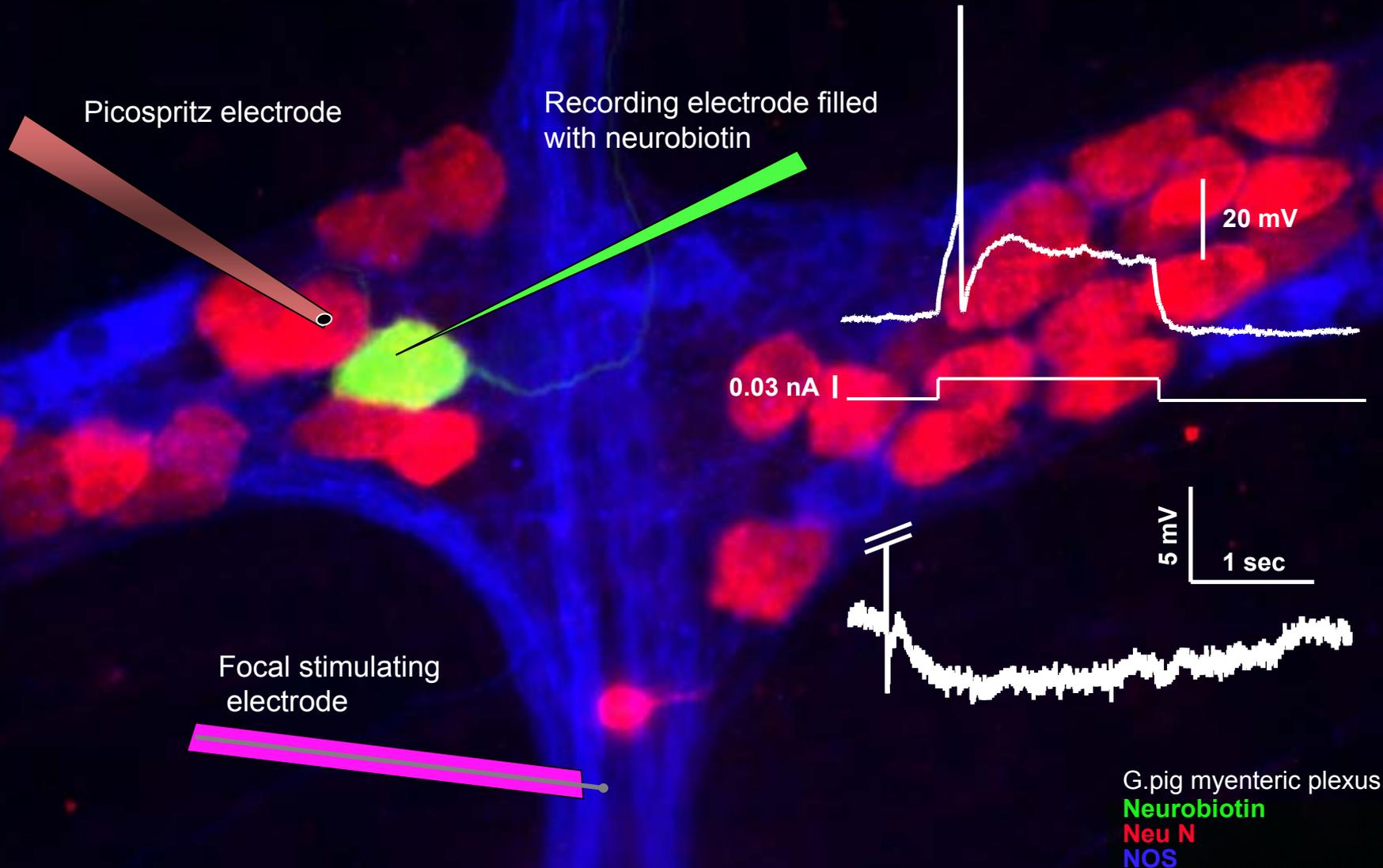
Human

	5-HT content	EC cell counts	5-HT release	5-HT transporter	References
TNBS-enteritis	↑	↑	↑	↓	Linden et al. (2003) AJP-GI 285:G207-G216
Trichinella-ileitis	?	↑	?	↓	Spiller et al., unpublished
Ulcerative colitis	↓	↓	No change	↓	Coates et al., in press
Campylobacter	?	↑	?	?	Spiller et al., unpublished

Enteritis-induced changes in reflex circuitry



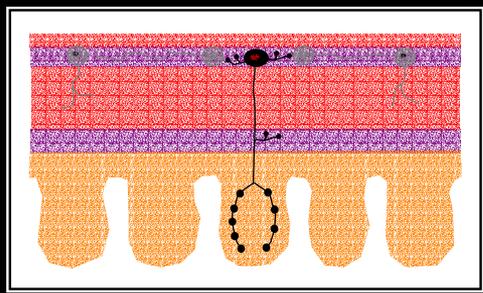
Use of combined technologies to elucidate the roles of various neurons in the ENS



Characteristics of Intrinsic Primary Afferent Neurons (AH neurons)

Dogiel Type II morphology

Resting membrane potential ~ -65 mV.



Prolonged afterhyperpolarization.

Rapid accommodation. *Shoulder on repolarizing phase of AP.*

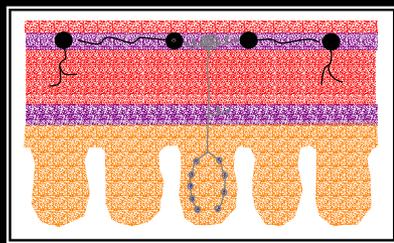
Spontaneous activity is rare.

Slow excitatory synaptic potentials are common, but fast synaptic potentials are rare.

Characteristics of Interneurons and Motor Neurons (S cells)

Dogiel Type I morphology

Resting membrane potential ~ -52 mV.



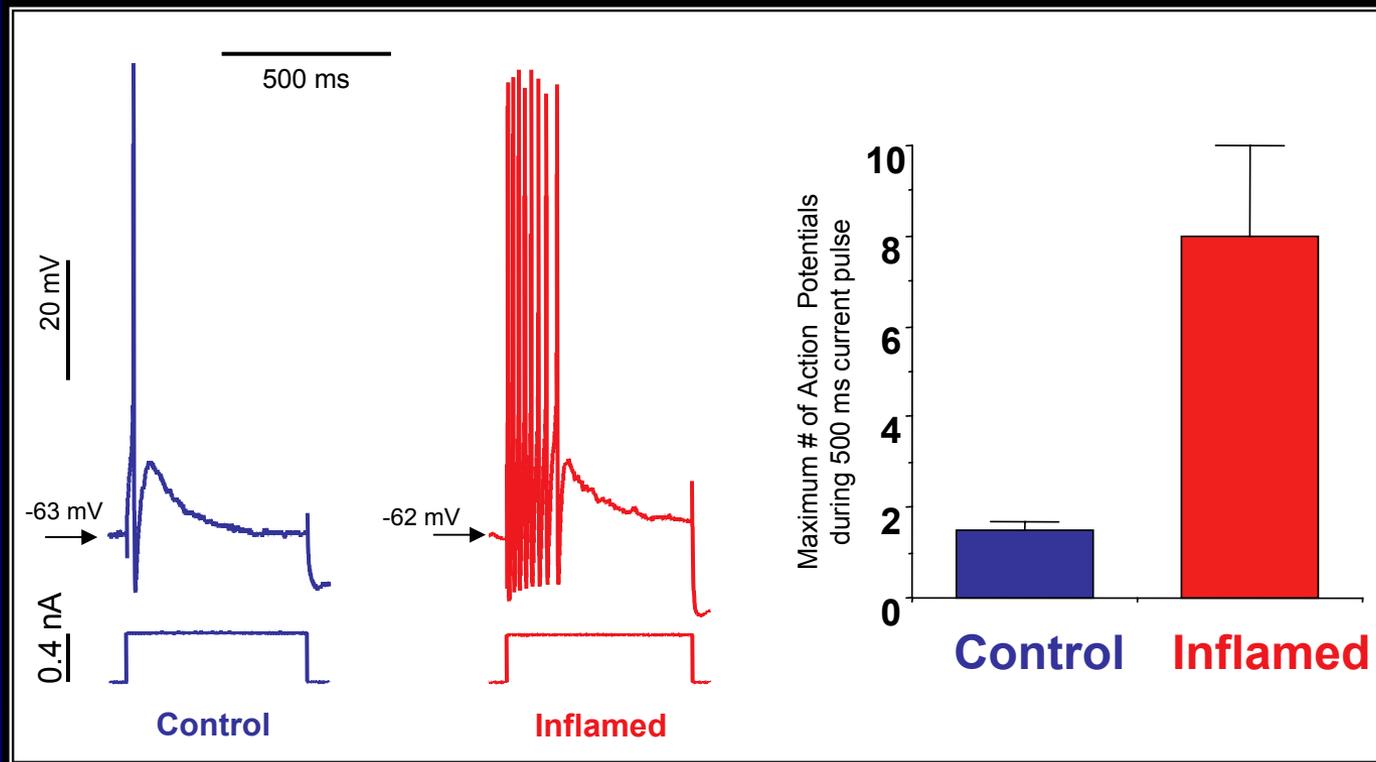
Brief afterhyperpolarization.

Slow accommodation.

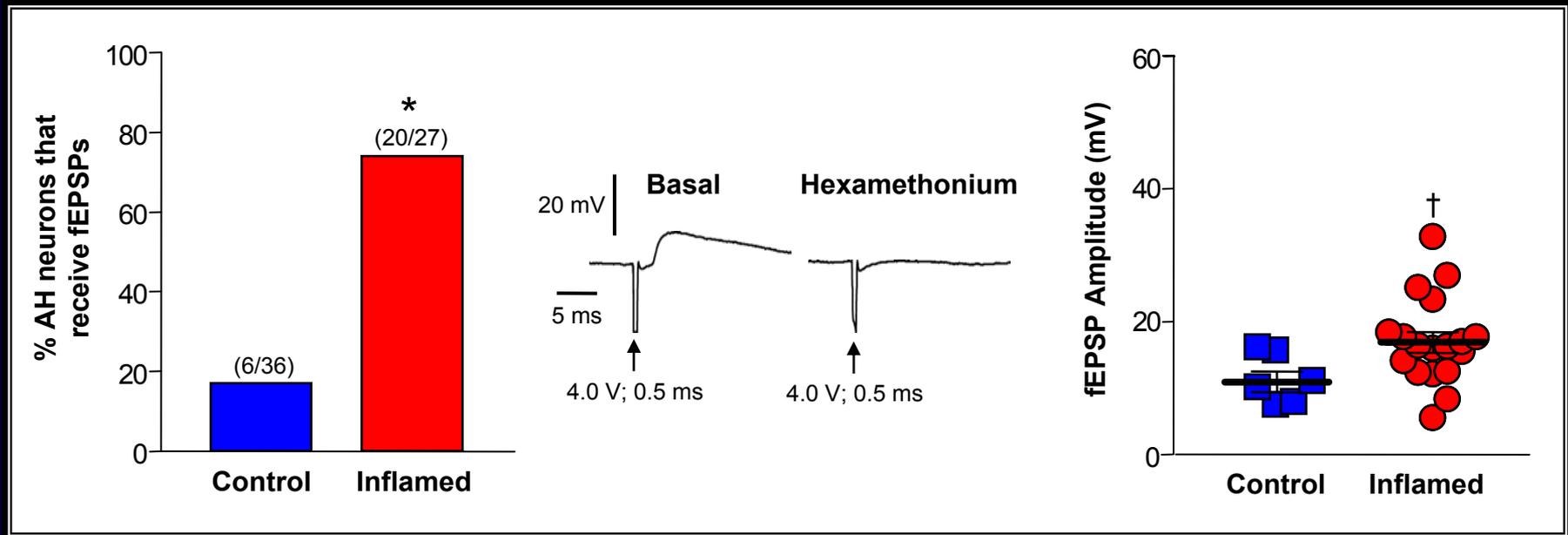
Spontaneous activity is common.

Slow and fast excitatory synaptic potentials are common.

AH neurons in the inflamed guinea pig colon are hyperexcitable



Fast excitatory synaptic input to AH neurons is augmented in TNBS colitis



Summary of TNBS colitis-induced changes in the electrical properties of AH neurons

Linden et al., 2003 J. Physiol. 547:589-601

	Resting Membrane Potential	Input resistance	# of Action Potentials	Spont. Activity	Anodal Break Potentials	AHP
TNBS colitis	—	—	↑↑	↑↑	↑↑	↓↓

Palmer et al., 1998 AJP. 275:G922-G935

<i>T. spiralis</i>	↓↓	↑↑	↑↑	↑↑	↑↑	↓↓
---------------------------	----	----	----	----	----	----

Summary of TNBS colitis-induced changes in S neurons (interneurons & motor neurons)

	Resting Membrane Potential	Input resistance	# of Action Potentials	Spont. Activity	Anodal Break Potentials	Fast EPSP	Slow EPSP
S neurons	—	—	—	—	—	↑↑	↑↑

What are the mechanisms of enhanced excitability in AH neurons of the inflamed colon?

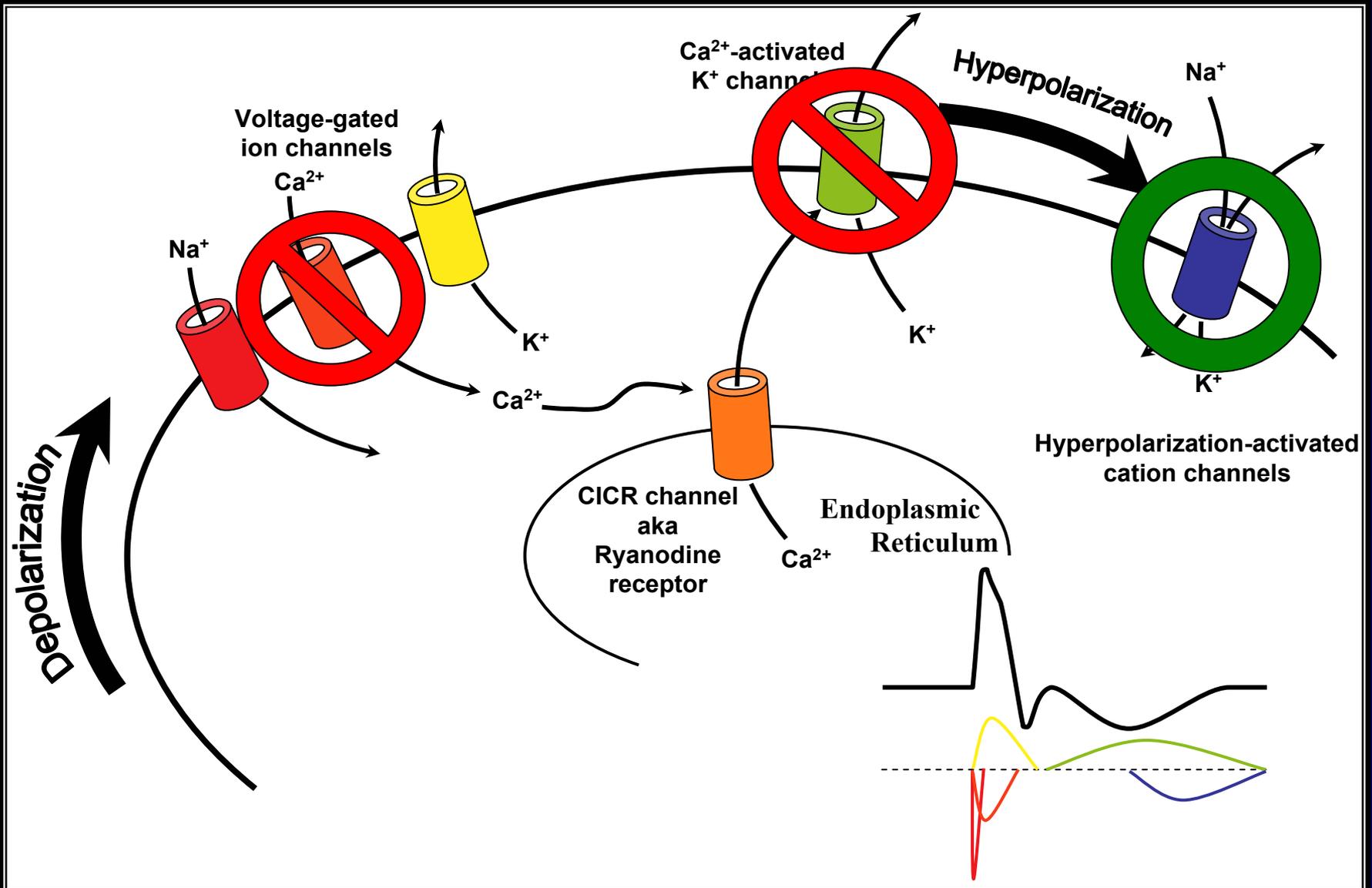
How and Why?



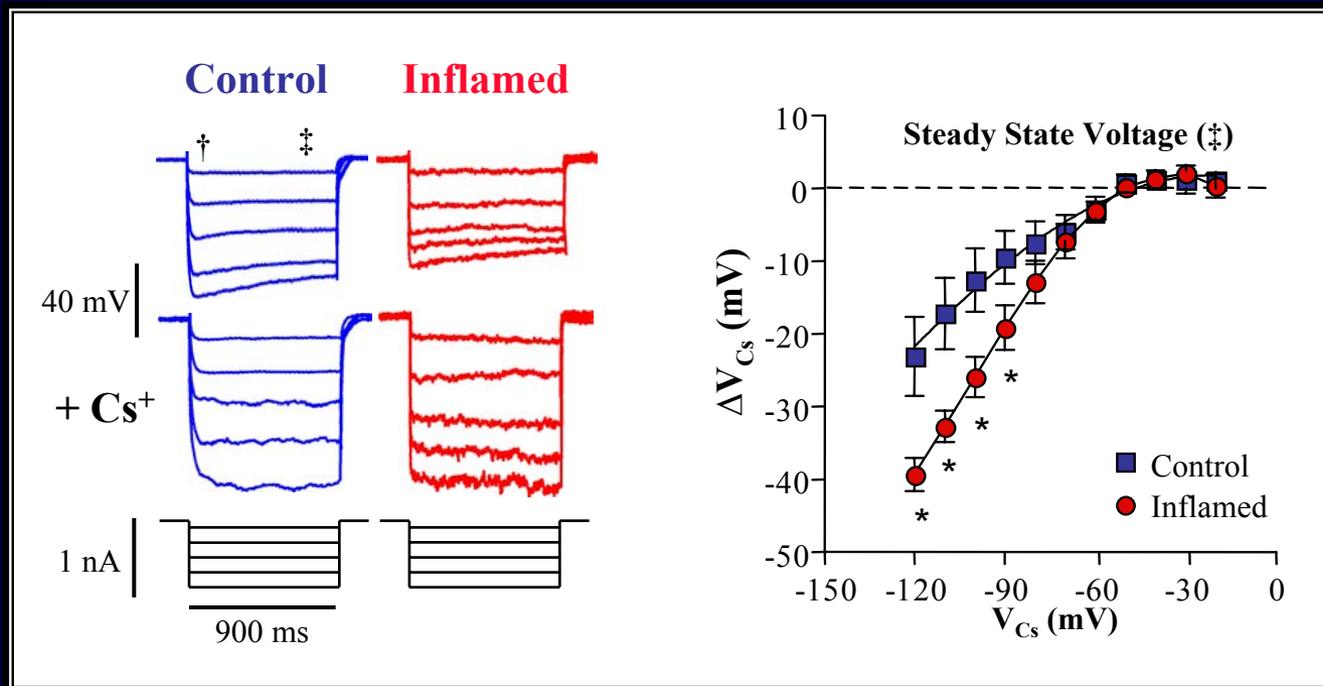
How does the inflammatory response lead to changes in IPAN excitability?

Why are the IPANs hyperexcitable?

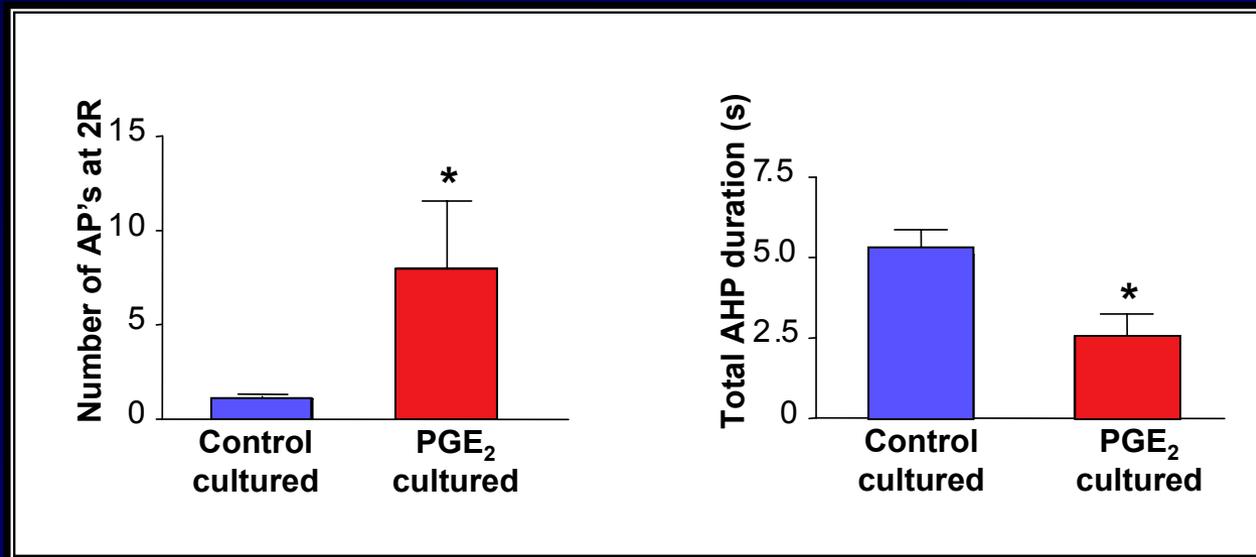
Ionic currents in AH Neurons



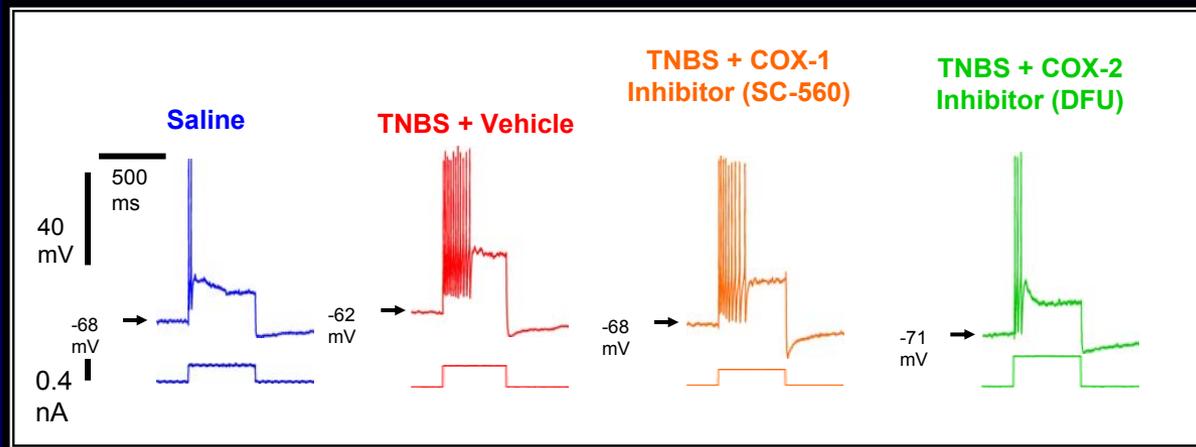
The Cs^+ -sensitive, hyperpolarization-activated current is enhanced in colitis



Prolonged exposure to PGE₂ leads to changes in AH neurons that are reminiscent of TNBS colitis



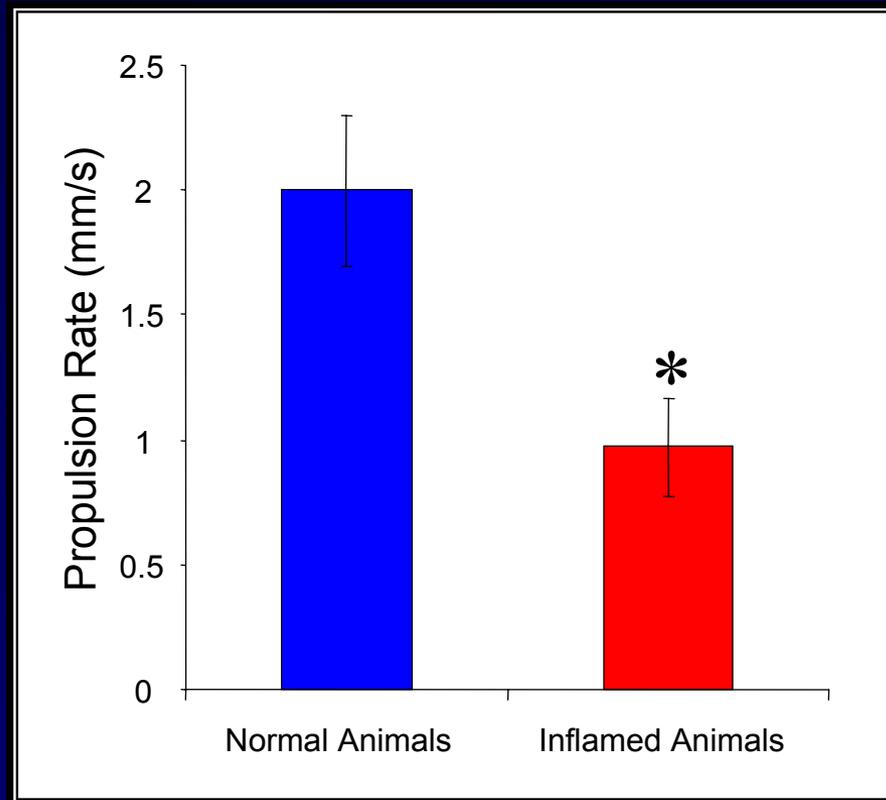
COX-2 activation contribute to hyperexcitability in AH neurons



Changes relative to control AH neurons

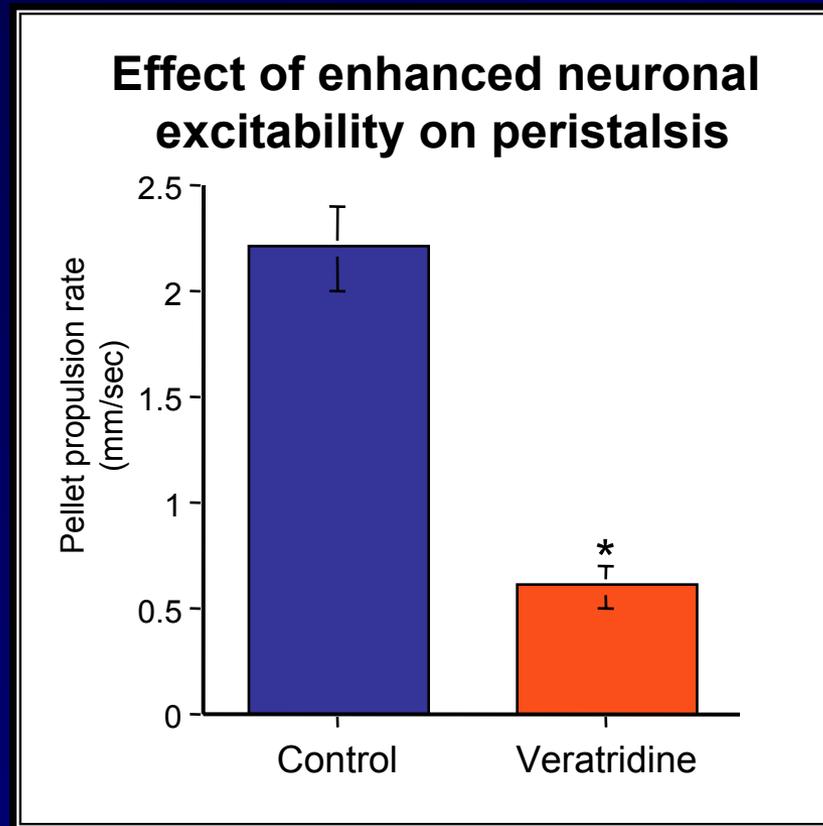
	Resting Membrane Potential	Input resistance	# of Action Potentials	Spont. Activity	Anodal Break Potentials	AHP
TNBS	—	—	↑↑	↑↑	↑↑	↓↓
TNBS + COX-1 blocker	—	—	↑↑	↑↑	↑↑	↓↓
TNBS + COX-2 blocker	—	—	—	—	—	—

Motility is impaired in the inflamed colon

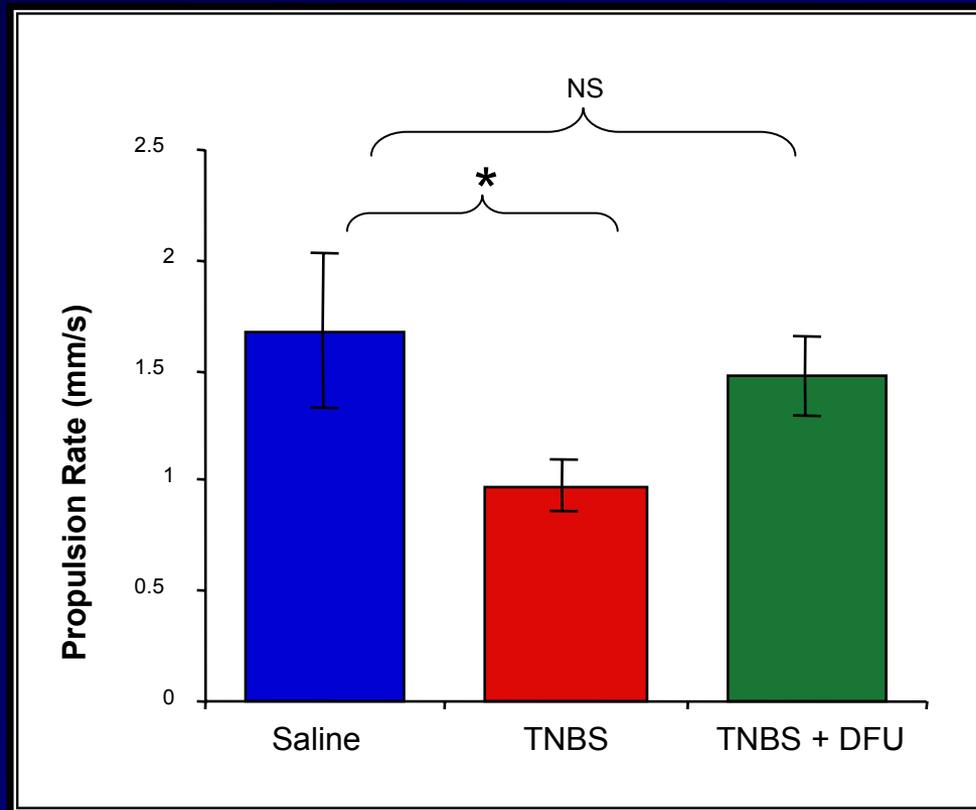


Can increased neuronal excitability lead to decreased propulsive motility?

Attention Deficit Disorder in the ENS

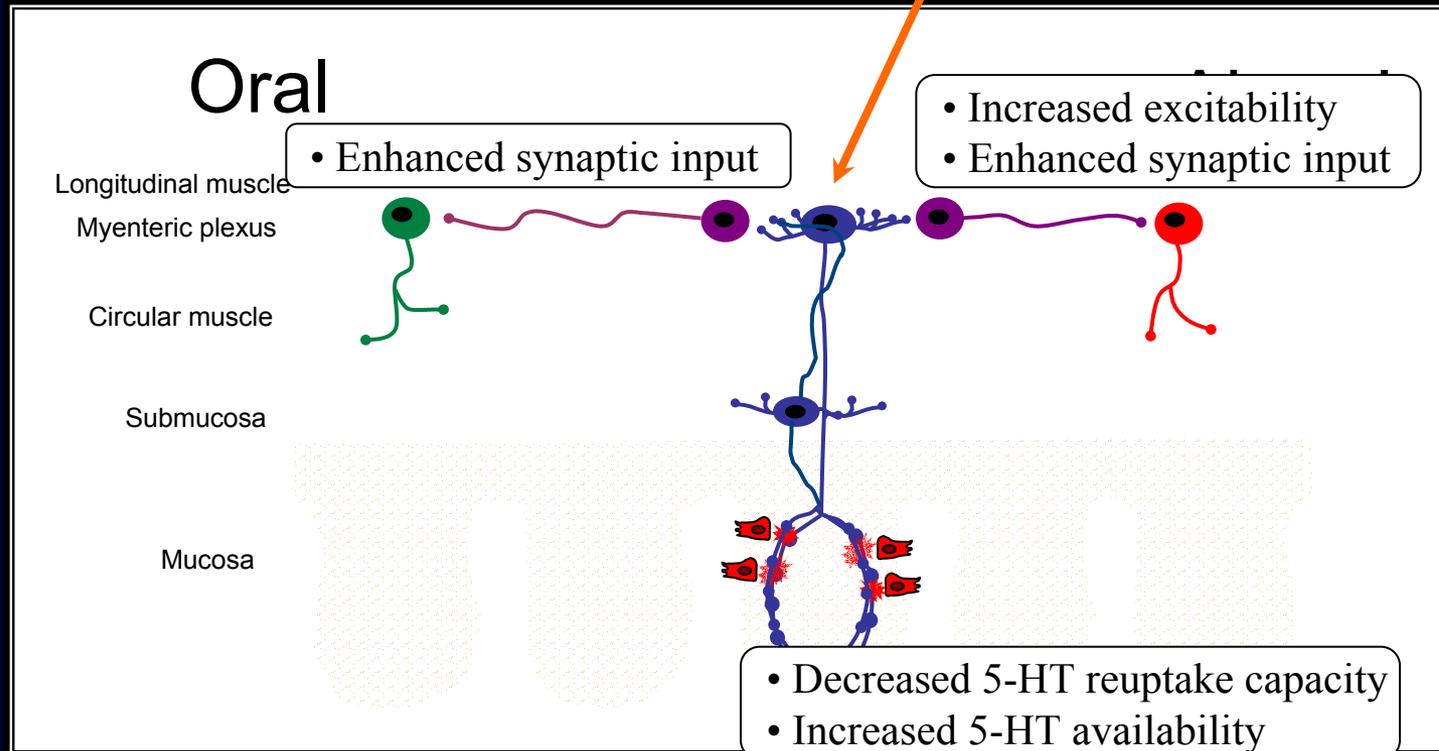


COX-2 blockade attenuates changes in motility that are associated with colitis

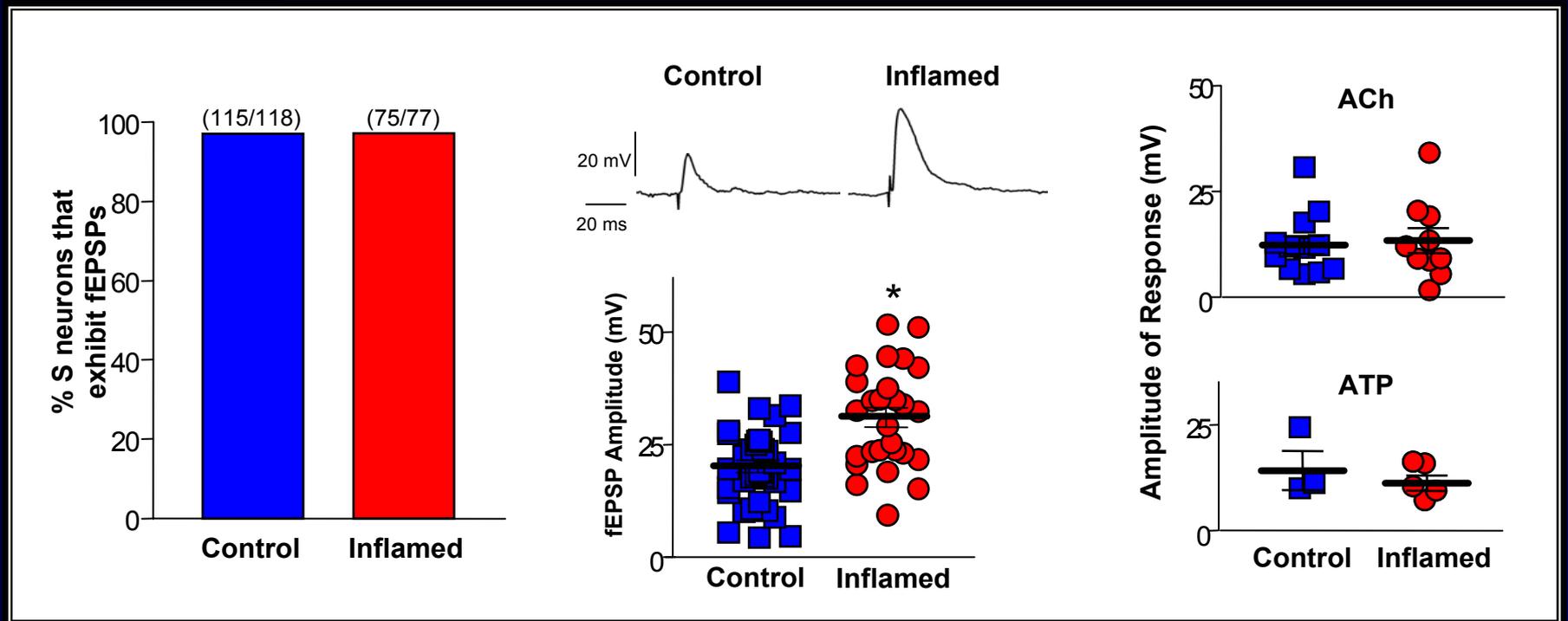


TNBS colitis-induced changes in reflex circuitry

- Increased excitability without altered RMP or IR
- Augmented I_h
- COX-2 activation is involved in elevated excitability
- Enhanced fast synaptic input



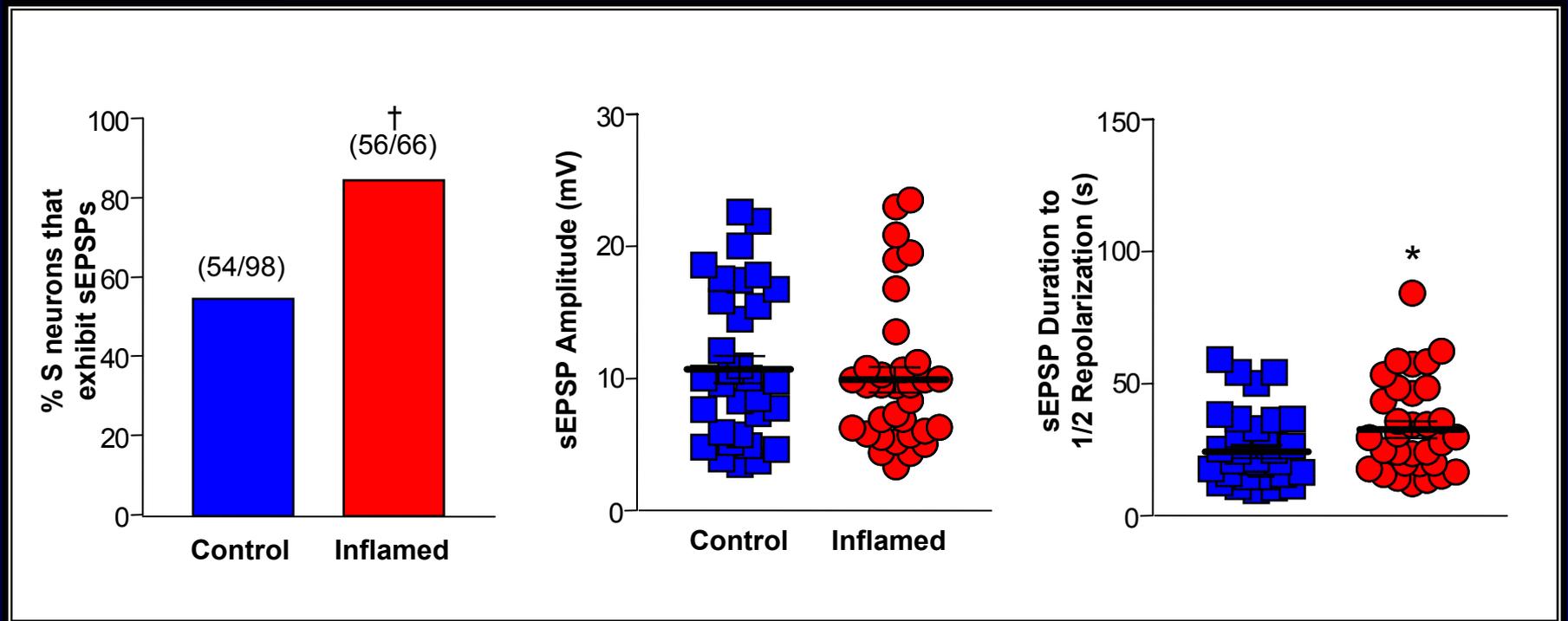
Fast excitatory synaptic input to S neurons is augmented in TNBS colitis



* $P < 0.001$

Linden, Sharkey & Mawe, unpublished

Slow excitatory synaptic input to S neurons is augmented in TNBS colitis



* $P < 0.05$; † $P < 0.01$